Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Virological Methods 129 (2005) 91–96
Journal of Virological Methods
www.elsevier.com/locate/jviromet

Shotgun sequencing of the negative-sense RNA genome of the rhabdovirus *Maize mosaic virus*

Sharon E. Reed[a,1], Chi-Wei Tsai[a], Kristen J. Willie[b], Margaret G. Redinbaugh[b], Saskia A. Hogenhout[a,*]

[a] *Department of Entomology, Ohio Agricultural Research and Development Center (OARDC), The Ohio State University, 1680 Madison Avenue, Wooster, OH 44691, USA*
[b] *USDA-ARS, Corn and Soybean Research and Department of Plant Pathology, The Ohio State University, Wooster, OH 44691, USA*

Received 7 January 2005; received in revised form 9 May 2005; accepted 16 May 2005
Available online 6 July 2005

Abstract

The maize-infecting nucleorhabdovirus, *Maize mosaic virus* (MMV), was sequenced to near completion using the random shotgun approach. Sequences of 102 clones from a cDNA library constructed from randomly-primed viral RNA were compiled into a 12,133 nucleotide (nt) contig containing six open reading frames. The contig consisted of 97 sequences averaging 660 bp in length. The average sequence coverage was six-fold, and 93% of the contig had sequence reads covering both strands. The remaining sequence was derived from single (5%) or multiple (2%) reads on the same strand. Three of the six ORFs showed significant similarities to the deduced protein sequences of the nucleocapsid, glycoprotein and polymerase sequences of other rhabdoviruses. The predicted gene order of the MMV genome was *3′-N-P-3-M-G-L-5′*. Shotgun sequencing of the MMV genome took approximately 127 h and cost $ 0.38 per nt (including labor), whereas the primer walking approach for sequencing the 13,782-nt MFSV genome [Tsai, C.-W., Redinbaugh, M.G., Willie, K.J., Reed, S., Goodin, M., Hogenhout, S. A., 2005. Complete genome gequence and in planta subcellular localization of maize fine streak virus proteins. J. Virol. 79, 5304–5314] took about 217 h and cost $ 0.50 per nt. Thus, the shotgun approach gave good depth of coverage for the viral genome sequence while being significantly faster and less expensive than the primer walking method. This technique will facilitate the sequencing of multiple rhabdovirus genomes.

1. Introduction

Rhabdoviruses are economically important pathogens that infect animals, humans, and plants (Hogenhout et al., 2003). The family *Rhabdoviridae* is comprised of six genera: *Lyssavirus* (contains *Rabies virus*); *Vesiculovirus* (*Vesicular stomatitis Indiana virus*); *Ephemerovirus* (*Bovine ephemeral fever virus*); *Novirhabdovirus* (*Infectious hematopoietic necrosis virus*); *Cytorhabdovirus* (*Lettuce necrotic yellows virus*); and *Nucleorhabdovirus* (*Sonchus yellow net virus*). Currently, 15 plant-infecting members of the *Rhabdoviridae* are listed in the Universal Database of the International Committee of Taxonomy of Viruses (ICTVdb; www.ncbi.nlm.nih.gov/ICTVdb/index.htm), and 88 possible plant rhabdoviruses are listed in the Virus Identification Data Exchange (VIDE) database (www.image.fs.uidaho.edu/vide). At this time, only six plant rhabdovirus genomes have been partially or completely sequenced. The lack of virus genome sequences has slowed the development of modern detection techniques and research on plant rhabdoviruses.

Current methods of genome sequencing of viruses, such as primer walking analysis of large cDNA clones, can be time consuming and hence costly in comparison to the more rapid shotgun sequencing methods that are currently used to sequence bacterial and eukaryotic genomes to completion. The random shotgun sequencing method involves the

* Corresponding author. Tel.: +1 330 263 3730; fax: +1 330 263 3686.
E-mail address: hogenhout.1@osu.edu (S.A. Hogenhout).
[1] Present address: University of Missouri, Life Science Center, Rm 414, 1201 Rollins St., Columbia, MO 65211, USA.

0166-0934/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jviromet.2005.05.013

cloning and sequencing of ~1000 bp genomic DNA fragments, and subsequent assembly of sequences into contigs based on sequence overlaps (Kaiser et al., 2003). Originally, Fleischmann et al. (1995) sequenced *Haemophilus influenzae* at 48 cents/bp using the shotgun method, but technological advances have lowered costs to 3–4 cents/bp for 99% of the genome sequence and 8–9 cents/bp for the complete genome (Fraser et al., 2002).

Here, we outline a procedure for the shotgun sequencing of the genome of *Maize mosaic virus* (MMV), a negative-sense RNA virus of the family *Rhabdoviridae*. MMV is a maize-infecting virus that is found in Central America, the Caribbean, India, Mauritius, Tanzania, and the United States (Jackson et al., 1981). It is transmitted in a persistent propagative manner by the planthopper *Peregrinus maidis* (Falk and Tsai, 1985; Ammar and Nault, 2002). MMV was classified as a nucleorhabdovirus because virions show the classic bullet-shape of rhabdoviruses and electron micrographs indicate that MMV viral particles bud through the perinuclear membrane (McDaniel et al., 1985; Ammar and Nault, 1985; Ammar, 1994). Three structural proteins were apparent after separation of MMV virion proteins by SDS-PAGE similarly to other members of the family *Rhabdoviridae* (Falk and Tsai, 1983). Results of this study showed that shotgun sequencing of a rhabdoviral genome is not only possible but is also an affordable and quick method relative to the more traditional primer walking method.

2. Materials and methods

2.1. MMV and RNA Isolation

MMV was maintained on maize seedlings by serial inoculations using viruliferous *P. maidis* as previously described (Falk and Tsai, 1985). Leaf laminar tissue (60 g) was collected from symptomatic maize plants approximately six weeks post inoculation and immediately homogenized in 3 vol. of extraction buffer containing 0.1 M sodium phosphate, pH 7.2 and 10% sucrose (Skaf and Carroll, 1995; Creamer, 1992). The homogenate was filtered through cheesecloth and miracloth then 5% (w/v) activated charcoal was added. After stirring for 20 min and repeating the filtration step, the homogenate was centrifuged at 3300 × *g* for 10 min at 4 °C. The resulting supernatant was centrifuged at 27,500 × *g* for 30 min at 4 °C. The resulting pellet was resuspended in 0.1 vol. extraction buffer, filtered through polyester fiber (Fairfield Corp., Danbury, CT), layered onto 25% (w/v) sucrose in 0.1 M sodium phosphate, pH 7.2, and centrifuged at 99,400 × *g* for 30 min at 4 °C. The pellet was resuspended in 6 ml 0.1 M sodium phosphate, pH 7.2, layered onto 10–40% (w/v) sucrose gradients in 0.1 M sodium phosphate, pH 7.2 (McDaniel et al., 1985), and then centrifuged at 112,700 × *g* for 25 min at 4 °C. The virus-containing milky band was extracted from the sucrose gradient, and diluted to 200 ml with 0.1 M sodium phosphate, pH 7.2. Virus particles were collected by centrifugation at 104,800 × *g* for 3 h at 4 °C. The pellet was resuspended in 500 μl PBS (0.1 MNaCl, 2.0 mM KCl, 10 mM Na_2HPO_4, 1.3 mM KH_2PO_4, pH 7.4).

RNA was isolated from the MMV virion preparations using the ToTALLY RNA isolation kit (Ambion, Austin, TX) and concentrations were measured using the RiboGreen RNA Quantitation Assay (Molecular Probes Inc., Eugene, OR) according to manufacturers' directions. Prior to library construction, the RNA was brought to 0.4 μg/ml glycogen (Invitrogen, Carlsbad, CA) and precipitated using sodium acetate and ethanol.

2.2. cDNA library construction

The SuperScript Choice System for cDNA Synthesis (Invitrogen Corp.) was used to construct a cDNA library from viral RNA following the manufacturer's instructions. Briefly, first strand cDNA was prepared from 4.4 μg total RNA by random priming with 125 ng random hexamers. After second strand cDNA synthesis, the double-stranded cDNA was ligated to 10 μg of *Eco*RI adapters, separated by column chromatography, and cloned into the *Eco*R1 site of calf intestinal phosphatase-treated pGEM4Z (Promega Corp., Madison, WI). Ligations were introduced into Top Ten One Shot F′ Chemically Competent *E. coli* cells (Invitrogen Corp.). Colonies were grown in 5 ml of Luria-Bertani Broth containing 50 μg/ml ampicillin (Sigma, St Louis, MO). Plasmids were isolated using the QIA Miniprep Spin Kit (Qiagen, Valencia, CA), and digested with *Eco*R1 and *Hin*dIII to determine insert presence and size. DNA concentrations were measured by PicoGreen dsDNA Quantitation Assay (Molecular Probes, Inc.), according to the manufacturer's directions.

2.3. Sequencing and sequence analysis

To initially evaluate library quality, ten colonies were selected to determine insert presence, and eight of these clones were sequenced from both directions. Subsequently, plasmids from an additional 100 clones were digested to determine the average insert size in the library. Of these, 94 plasmid inserts were sequenced from both directions. Sequencing was performed at the Plant-Microbe Genomics Facility at The Ohio State University (Columbus, Ohio) using an ABI 3700 DNA Sequence Analyzer and Big Dye Terminator Cycle Sequencing chemistry (Applied Biosystems Inc., Foster City, CA). Sequence quality scores were obtained using McPhred/McPhrap (CodonCorp Corp., Dedham, MA). Removal of low quality and vector sequence, and contig assembly were conducted with Sequencher (Gene Codes Corp., Ann Arbor, MI). At the 5′ end, sequences were trimmed until the first 15 bases contained less than three bases with confidence levels less than 20. At the 3′ end, sequences were trimmed until the last 15 bases contained less than three bases with confidence levels less than 20. The average length was calculated for good quality reads of greater than 100 bp. Subsequently these reads were assembled into contigs using

Table 1
Results of shotgun library construction, sequencing and assembly

Number of clones in the MMV cDNA library	9065
Average insert size (bp)[a]	1500 ± 780
Number of inserts sequenced from both directions	102
Number of good quality sequence reads	163
Number of failed reactions	41
Average good quality sequence read length (bp)	704 ± 194
Total number of contigs	4
Sequence reads assembled into the MMV contig	97
Sequence reads that assembled into other contigs	62
Sequence reads that did not assemble into contigs	5
MMV contig length after assembly (bp)	12,133
MMV genome depth of coverage	Six-fold

[a] The average size of the insert was calculated based on fragments released after digestion of plasmids with *Eco*RI and *Hin*dIII. Data presented are the mean ± S.D.

the Dirty Data algorithm with a minimum match of 85% and a minimum overlap of twenty. The average length of good quality reads that assembled into contigs was calculated.

Open reading frame (ORF) analysis was performed using both MacVector 6.5.3 (Oxford Molecular Group, 1999) and the ORF Finder database at the NCBI website. The six ORFs identified in the MMV genome were compared, using BlastX, to the non-redundant (nr) protein database of GenBank. The MMV genome sequence was submitted to GenBank under accession number AY618418.

3. Results and discussion

3.1. cDNA library construction and analysis of sequences

MMV virions from three independent isolations totaling 2.4 mg protein were combined for RNA isolation generating 11 μg of total RNA. Of this, 4.4 μg were used for randomly primed cDNA library construction. The cDNA library yielded 9065 clones with an average insert size of 1500 (standard deviation (S.D.) = 780 bp) based on *Eco*R1 and *Hin*dIII digestions of 100 plasmids (Table 1). Initially, inserts of eight plasmids were sequenced from both directions using SP6 and T7 primers, and of these, six insert sequences had similarities to genome sequences from other rhabdoviruses. Subsequently, 94 more clones from the MMV cDNA library were sequenced from the forward (SP6 primer) and reverse (T7 primer) directions.

After removal of low quality sequence and vector sequences, 163 of the 204 sequences (80%) contained reads of more than 100 bp, and were used for further analysis (Table 1). Thus, only 20% of the sequence reads were below 100 bp after trimming the vector sequences. The average length of a read was 704 (S.D. = 194 bp).

Assembly of the insert sequences of more than 100 bp resulted in four contigs made up of 158 sequences and five singletons. The largest contig of 12,133 bp consisted of 97 sequence reads with an average length of 660 bp (S.D. = 180 bp). A BlastX analysis of this contig against the nr protein database of GenBank revealed significant similarity to the polymerase (L) protein of the plant rhabdoviruses: *Rice yellow stunt virus* (RYSV) (*E*-value: 0.00), *Sonchus yellow net virus* (SYNV) (*E*-value: e^{-168}) and *Northern cereal mosaic virus* (NCMV) (*E*-value: e^{-130}), indicating that the contig represented the MMV genome sequence. This 12,133 nt sequence of the MMV genome had a six-fold average coverage, and both strands were sequenced for 93% of the contig, while 5.0% of the sequence was derived from single reads of one strand and 2.0% was derived from multiple sequences on the same strand (data not shown).

Five singlet sequences remained after contig formation. BlastX analysis of the nucleotide sequences against GenBank nr database revealed that three singlets (635, 669 and 732 bp) were most similar to a ferritin like protein (*E*-value: $8e^{-69}$), an *E. coli* hypothetical protein (*E*-value: $9e^{-35}$), and the L protein of MMV (*E*-value: $2e^{-78}$), respectively, while the remaining two singlets had no significant similarities to the database. BlastX searches on three other smaller contigs (1587, 1,700, and 2968 bp) indicated a high level similarity with an rRNA intron encoding an endonuclease of *Oryza sativa* (*E*-value: $3e^{-24}$), MMV L protein (*E*-value: 0.0), and cytochrome P450 monooxygenase of *Zea mays* (*E*-value: e^{-130}), respectively. These three contigs were assembled from 62 sequences (37%) of the 163 sequences of more than 100 bp in length. Thus, one singlet and one small contig contained fragments of the MMV genome sequence. To investigate why these two were not assembled into the larger contig, these sequences were examined further and revealed that the singlet was derived from a chimeric insert composed of a segment of the MMV genome and a plant gene, and the contig sequence was most likely derived from a misassembly as it was missing a fragment corresponding to nucleotides 11,171 to 11,600 of the 12,133 bp contig.

3.2. Annotation of the MMV genome sequence

Six ORFs located on the antigenomic strand were identified in the 12,133 bp MMV contig (Fig. 1) and showed a genome organization similar to those of other rhabdoviruses (Table 2). The ORF sequences were compared with the GenBank nr protein database using BlastX. The sequence of the largest ORF (ORF6), located at the most distal portion of

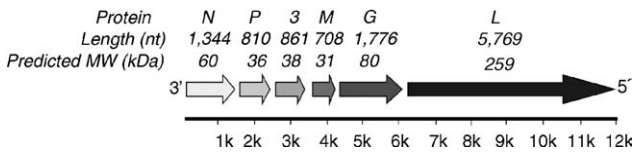

Fig. 1. Schematic representation of the genome organization of *Maize mosaic virus*. Figure details were derived from MacVector using the sequences assembled with Sequencher. Abbreviations: N, nucleocaspid protein; P, phosphoprotein; 3, deduced protein of open reading frame 3; M, matrix protein; G, glycoprotein; and L, polymerase protein; nt, nucleotides; MW, molecular weight.

Table 2
Comparison of the genome organizations of the livestock pathogen VSIV, and the plant infecting nucleorhabdoviruses and cytorhabdoviruses

Virus	Gene order										
VSIV	3′	*N*	*P*					*M*	*G*		*L* 5′
Nucleorhabdovirus											
MMV	3′	*N*	*P*	*3*				*M*	*G*		*L* 5′
MFSV	3′	*N*	*P*	*3*	*4*			*M*	*G*		*L* 5′
SYNV	3′	*N*	*P*		*sc4*			*M*	*G*		*L* 5′
RYSV	3′	*N*	*P*	*3*				*M*	*G*	*6*	*L* 5′
Cytorhabdovirus											
LNYV	3′	*N*	*P*		*4b*			*M*	*G*		*L* 5′
NCMV	3′	*N*	*P*	*3*	*4*	*5*	*6*	*M*	*G*		*L* 5′

Abbreviations: VSIV, Vesicular stomatitis *Indiana virus*; MMV, *Maize mosaic virus*; MFSV, *maize fine streak virus*; SYNV, *Sonchus yellow net virus*; RYSV, *Rice yellow stunt virus*; LYNV, *Lettuce necrotic yellows virus*; and NCMV, *Northern cereal mosaic virus*.

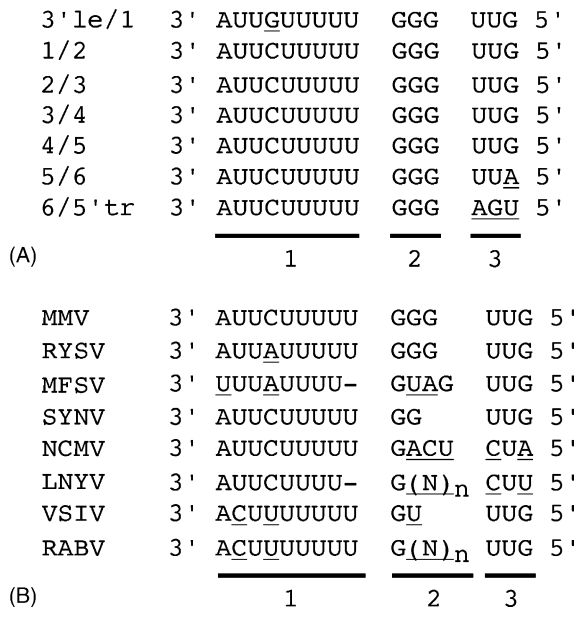

Fig. 2. Comparative sequence analysis of gene junctions of rhabdovirus genomes. (A) Gene junctions of the MMV genome. The three motifs are indicated and correspond to the 3′ end of the mRNAs (1), intergenic sequences (2), and the 5′ end of the mRNAs (3). (B) Consensus sequences of gene junctions of plant and animal rhabdoviruses. All sequences are presented in genomic sense in the 3′ to 5′ orientation, and nucleotide differences are underlined. Abbreviations: 3′ le, 3′ leader sequence; 5′ tr, 5′ trailer sequence; (N)n, nucleotide type and number may vary. RABV, rabies virus.

the genome, was most similar to the L protein of RYSV and SYNV (*E*-values: 0.0), and ORF5 was most similar to the glycoproteins (G) of RYSV (*E*-value: $2e^{-22}$) and SYNV (*E*-value: 0.072). ORF1 was most similar to the nucleocapsid proteins (N) of RYSV (*E*-value: $5e^{-48}$) and maize fine streak virus (MFSV) (*E*-value: $3e^{-30}$). The deduced protein sequences of the remaining three ORFs were not closely related to any proteins in GenBank. However, based on gene order in other rhabdoviruses and ORF size (Redinbaugh and Hogenhout, 2005), ORF2 likely encodes the phosphoprotein (P) and ORF4 possibly encodes the matrix protein (M). Thus, the most likely gene order of the MMV genome was *N*, *P*, *3*, *M*, *G* and *L* (Fig. 1). Nucleotide lengths of these MMV ORFs were 1344 nt for N, 810 nt for the tentative P, 861 nt for ORF 3, 708 nt for the tentative M, 1776 nt for G, and 5769 nt for L. The calculated molecular weights of the proteins based on the deduced protein sequences would be ~60 kDa for N, ~36 kDa for P, ~38 kDa for the ORF3 protein, ~31 kDa for M, ~80 kDa for G, and ~259 kDa for L.

The basic gene order of a rhabdovirus genome is 3′ leader, *N*, *P*, *M*, *G*, *L*, and the 5′ trailer (Hogenhout et al., 2003). Thus, MMV had an additional gene, *ORF3*, located between the *P* and *M* genes. The genome structure of MMV most closely matched those of SYNV and *Lettuce necrotic yellows virus* (LNYV), which also have an additional ORF between the *P* and *M* genes (Table 2). In SYNV, this additional gene is known as *sc4*, which is involved in cell-to-cell movement in plants (Scholthof et al., 1994), and this may also be the role of the ORF3 protein product of MMV.

Interestingly, the genome organization of MMV was different from those of two other cereal-infecting nucleorhabdoviruses, MFSV and RYSV (Table 2). MFSV contained two extra genes, *3* and *4*, which were expressed in MFSV-infected plants (Tsai et al., 2005). The RYSV genome sequence also contained an additional ORF, gene *3*, between the *P* and *M* genes, and in addition had a second additional ORF, gene *6*, located between the *G* and *L* genes (Huang et al., 2003). The RYSV P6 is a phosphorylated structural protein that is expressed in insects, but not in plants (Huang et al., 2003) suggesting a role of this protein in insect transmission. Insect specific expression of other plant rhabdovirus sequences remains to be tested.

Clones corresponding to the 3′ and 5′ termini of the MMV genome were not sequenced with the shotgun approach. By comparison to other nucleorhabdoviruses (RYSV, SYNV, and MFSV), it is likely that the MMV genome sequence lacks 20–80 nt of the leader sequence at the 3′end and 30–100 nt of trailer sequence at the 5′ end. The inefficiency of cloning the genomic termini may not be surprising as other plant rhabdoviruses have potentially stable secondary structures at the 5′ and 3′ ends of the genomic RNA (Choi et al., 1994; Wetzel et al., 1994; Tanno et al., 2000; Tsai et al., 2005). We will determine the terminal sequences of MMV by RACE (Tsai et al., 2005).

The gene junction sequences between rhabdovirus ORFs contain a conserved region that correspond to the polyadenylation signature at the end of a gene sequence, the intergenic region and the transcriptional start site of the next gene. For MMV, the conserved gene junction sequence was 3′-AUUCUUUUUGGGUUG-5′ (Fig. 2A), and was similar to those of animal and other plant rhabdoviruses (Fig. 2B). Among rhabdoviruses, the polyadenylation signatures consist of 8–9 nucleotides of $(U)_n$ interspersed with an occasional A or a C, the intergenic regions are most variable in length and sequence but usually contain one or more Gs, and the transcription start sites contain the three consensus nucleotides, UUG, except for the two cytorhabdoviruses (NCMV and

Table 3
Time comparison of the shotgun and primer walking sequencing procedures

	Shotgun (MMV)[a]	Primer walking (MFSV)[a,b]
Virus isolation	24	24
cDNA library construction	25.5	25.5
First round of colony picking and library quality analysis	7.5	7.5
First round of sequence analysis	4	20
Colony picking and plasmid isolation for the 96-well plates	46	NA
Sequence and assembly of sequences from 96-well plates	20	NA
Design of primers for five rounds of primer walking steps	NA	25
Cloning of PCR products	NA	15
Sequence analysis and assembly of sequences of a total of 12 cDNA clones and 4 PCR products in the five primer walking steps	NA	100
Total hours	127	217

[a] Time is indicated in hours.
[b] The MFSV genome sequence is described in Tsai et al., 2005. NA, not applicable.

LNYV), which have CUA and CUU transcription start sites (Fig. 2B). As expected, the MMV gene junction sequence was most similar to those of RYSV and SYNV (Fig. 2B).

3.3. Time and cost analysis of the shotgun and primer walking sequencing procedures

To determine the effectiveness of the shotgun sequencing of a viral genome, the time and cost of the shotgun sequencing procedure of the MMV genome was compared to that of the slightly larger MFSV genome (Tables 3 and 4), which was recently sequenced using the traditional primer walking method (Tsai et al., 2005). Shotgun sequencing of the MMV genome took a total of 127 h, whereas primer-walking sequencing of the MFSV genome took 217 h (Table 3). For cost analysis, personnel costs were calculated at $15.86/h including employee benefits as 30% of salary. Supply costs were derived from a lab supply budget of $10,000 per 260-day (2080 h) work year multiplied by work hours, and did not include computer programs or large equipment used in the project since none were purchased for this purpose. Further, costs of the cDNA library kit, the plasmid vector, and the competent *E. coli* cells were calculated separately under the item "cDNA library costs" that were the same for the shotgun and primer walking methods. The supplies costs for MFSV also included $ 350 for primers and $50 for PCR cloning materials. Sequencing costs included costs for 204 reactions and was higher for the MFSV genome, because the price for individual sequence reaction is $10/reaction as opposed to $8/reaction for a 96 well plate. The total cost to sequence the 12,133-nt MMV and 13,782-nt MFSV genomes was $0.38 and 0.51, respectively (Table 4).

In summary, in this manuscript we show that shotgun sequencing of a rhabdovirus genome is possible while being an affordable and quick method in comparison to the traditional primer walking method and, in addition, gives a good depth of genome coverage. The shotgun sequencing allowed for quick and almost complete coverage of the MMV genome although MMV is difficult to purify and MMV preparations had significant plant contamination. At this time, only six of the ~90 plant rhabdovirus genomes have been partially or completely sequenced. The shotgun sequencing technique may facilitate the sequencing of more rhabdovirus genomes. This genome sequence information will lead to a better understanding of rhabdovirus evolution and factors that determine the host range of these viruses. This is important, because despite their small genomes, rhabdoviruses are pathogens of humans, animals, insects and plants (Hogenhout et al.,

Table 4
Cost comparison of the shotgun and primer walking sequencing procedures

	Shotgun (MMV)	Primer walking (MFSV)
Personnel cost, including 30% benefits ($15.86/h) ($)	2014	3442
Cost of supplies ($10,000 for 260 work days (2080 h per year) ($)	611	1043
Cost of cDNA library construction[a] ($)	265	265
Cost of sequencing[b] ($)	1696	1670
50 Primers	NA	350
PCR cloning materials	NA	50
Total cost ($)	4586	6820
Sequencing cost per base[c]	0.38	0.50

[a] Includes the costs of one reaction of the cDNA library kit, the plasmid vector, and the competent *E. coli* cells.
[b] The sequence costs for MMV includes 96 inserts sequenced from both directions (192 reactions) at $8/reaction for the 96 well plate and eight inserts from both directions (16 reactions) at $10/reaction, and the sequence costs for MFSV includes 167 reactions (i.e. 132 good quality reads, and 35 unsuccessful sequence reactions) at $10/reaction.
[c] Genome lengths excluding the 5′ and 3′ ends were 12,133 nt for MMV (this study) and 13,782 nt for MFSV (Tsai et al., 2005); NA, not applicable.

2003). Further, arthropods, such as mosquitoes, flies and plant-feeding insects, transmit many rhabdoviruses and may initiate new outbreaks by introducing rhabdoviruses among vertebrate and plant hosts.

Acknowledgements

We thank Angela Strock and John Abt for maintaining *P. maidis* populations and MMV-infected plant material. This project was supported by the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service, grant number 2002-35302-12653.

References

Ammar, E.D., 1994. Propagative transmission of plant and animal viruses by insects: factors affecting vector specificity and competence. In: Harris, K.F. (Ed.), Advances in Disease Vector Research, Vol. 10. Springer-Verlag, New York, Inc., pp. 289–331.

Ammar, E.D., Nault, L.R., 1985. Assembly and accumulation sites of Maize mosaic virus in its planthopper vector. Intervirology 24, 33–41.

Ammar, E.D., Nault, L.R., 2002. Virus transmission of leafhoppers, planthoppers, and treehoppers (Auchenorrhyncha Homoptera). Adv. Bot. Res. 36, 141–167.

Choi, T.J., Wagner, J.D., Jackson, A.O., 1994. Sequence analysis of the trailer region of sonchus yellow net virus genomic RNA. Virology 202, 33–40.

Creamer, R., 1992. Purification and protein characterization of sorghum stunt mosaic rhabdovirus. Phytopathology 82, 1473–1476.

Falk, B.W., Tsai, J.H., 1983. Physiochemical properties of maize mosaic virus. Phytopathology 73, 1536–1539.

Falk, B.W., Tsai, J.H., 1985. Serological detection and evidence for multiplication of maize mosaic virus in the planthopper *Peregrinus maidis*. Phytopathology 75, 852–855.

Fleischmann, R.D., Adams, M.D., White, O., Clayton, R.A., Kirkness, E.F., Kerlavage, A.R., Bult, C.J., Tomb, J.F., Dougherty, B.A., Merrick, J.M., McKenney, K., Sutton, G., Fitzhugh, W., Fields, C., Gocayne, J.D., Scott, J., Shirley, R., Liu, L.I., Glodek, A., Kelley, J.M., Weidman, J.F., Phillips, C.A., Spriggs, T., Hedblom, E., Cotton, M.D., Utterback, T.R., Hanna, M.C., Nguyen, D.T., Saudek, D.M., Brandon, R.C., Fine, L.D., Fritchman, J.L., Fuhrmann, J.L., Geoghagen, N.S.M., Gnehm, C.L., McDonald, L.A., Small, K.V., Fraser, C.M., Smith, H.O, Venter, J.C., 1995. Whole genome sequencing and assembly of *Haemophilus influenzae*. Science 269, 496–512.

Fraser, M.F., Eisen, J.A., Nelson, K.E., Paulsen, I.T., Salzberg, S.T., 2002. The value of complete microbial genome sequencing (You get what you pay for). J. Bacteriol. 184, 6403–6405.

Hogenhout, S.A., Redinbaugh, M.G., Ammar, E-D., 2003. Plant and animal rhabdovirus host range: a bug's view. Trends Microbiol. 11, 264–271.

Huang, Y., Heng, Z., Luo, Z., Chen, X., Fang, R-X., 2003. Novel structure of the genome of *Rice yellow stunt virus*: identification of the gene 6-encoded virion protein. J. Gen. Virol. 84, 2259–2264.

Jackson, A.O., Milbrath, J.M., Jedlinski, H., 1981. Rhabdovirus diseases of the Graminea. In: Gordon, D.T., Knoke, J.K., Scott, G.E. (Eds.), Virus and Virus like Diseases of Maize in the United States. Southern Cooperative Series Bulletin 247, Ohio Agricultural Resource and Development Center, Wooster, Ohio, pp. 51–76.

Kaiser, O., Bartels, D., Bekel, T., Goesmann, A., Kespohl, S., Puhler, A., Meyer, F., 2003. Whole genome shotgun sequencing guided by bioinformatics pipelines – an optimized approach for an established technique. J. Biotechnol. 106, 121–133.

McDaniel, L.L., Ammar, E.-D., Gordon, D.T., 1985. Assembly, morphology, and accumulation of a Hawaiian isolate of maize mosaic virus in maize. Phytopathology 75, 1167–1172.

Redinbaugh, M. G., Hogenhout, S.A. 2005. Plant rhabdoviruses. In: Fu, Z., Koprowski, H., (Eds.), The World of Rhabdoviruses. Current Topics in Microbiology and Immunology, Vol. 292, 143–153.

Scholthof, K.G., Hillman, B.I., Modrell, B., Heaton, L.A., Jackson, A.O., 1994. Characterization and detection of sc4: A sixth gene encoded by sonchus yellow net virus. Virology 204, 279–288.

Skaf, J.S., Carroll, R.T., 1995. Purification of barley yellow streak mosaic virus and detection by DAS-ELISA and ISEM using polyclonal antibodies. Plant Dis. 79, 1003–1007.

Tanno, F., Nakatsu, A., Toriyama, S., Kojima, M., 2000. Complete nucleotide sequence of northern cereal mosaic virus and its genome organization. Arch. Virol. 145, 1373–1384.

Tsai, C-W., Redinbaugh, M.G., Willie, K.J., Reed, S., Goodin, M., Hogenhout, S.A., 2005. Complete genome gequence and in planta subcellular localization of maize fine streak virus proteins. J. Virol. 79, 5304–5314.

Wetzel, T., Dietzgen, R.G., Dale, J.L., 1994. Genomic organization of lettuce necrotic yellows rhabdovirus. Virology 200, 401–412.